Short communication

Tenofovir use is associated with an increase in serum alkaline phosphatase in the Swiss HIV Cohort Study

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Background: Tenofovir (TDF) use has been associated with proximal renal tubulopathy, reduced calculated glomerular filtration rates (cGFR) and losses in bone mineral density. Bone resorption could result in a compensatory osteoblast activation indicated by an increase in serum alkaline phosphatase (sAP). A few small studies have reported a positive correlation between renal phosphate losses, increased bone turnover and sAP.

Methods: We analysed sAP dynamics in patients initiating (n=657), reinitiating (n=361) and discontinuing (n=73) combined antiretroviral therapy with and without TDF and assessed correlations with clinical and epidemiological parameters.

Results: TDF use was associated with a significant increase of sAP from a median of 74 U/l (interquartile range 60–98) to a plateau of 99 U/l (82–123) after 6 months (P<0.0001), with a prompt return to baseline upon TDF discontinuation. No change occurred in TDF-sparing regimes. Univariable and multivariable linear regression analyses revealed a positive correlation between sAP and TDF use (P≤0.003), but no correlation with baseline cGFR, TDF-related cGFR reduction, changes in serum alanine aminotransferase (sALT) or active hepatitis C.

Conclusions: We document a highly significant association between TDF use and increased sAP in a large observational cohort. The lack of correlation between TDF use and sALT suggests that the increase in sAP is because of the bone isoenzyme and indicates stimulated bone turnover. This finding, together with published data on TDF-related renal phosphate losses, this finding raises concerns that TDF use could result in osteomalacia with a loss in bone mineral density at least in a subset of patients. This potentially severe long-term toxicity should be addressed in future studies.

Introduction

Tenofovir (TDF) is a first-line antiretroviral agent with a favourable safety profile. However, it has been associated with proximal renal tubulopathy (PRT) [1–5], reduced calculated glomerular filtration rates (cGFR) [3,6–8] and losses in bone mineral density [9,10].

TDF is partially eliminated via the proximal tubule, where it is thought to accumulate and to impair the selective reabsorption of glomerular filtrate. TDF-related renal phosphate and calcium losses are particularly important, as they could result in the compensatory resorption of normally-structured bone (that is, osteoporosis) and mineralization defects in regenerating bone (that is, osteomalacia). Bone regeneration is brought about by activated osteoblasts and thus can be evidenced by an increase in serum alkaline phosphatase (sAP). Four small studies have reported a positive correlation between renal phosphate losses, bone turnover and sAP [10–13].

We analysed sAP dynamics relative to TDF use in a large observational cohort and evaluated the association

with TDF-related nephrotoxicity as evidenced by a reduction in cGFR.

Methods

The study participants were recruited from a population we had identified to study TDF-related nephrotoxicity [8] in the Swiss HIV Cohort Study (SHCS). We included patients starting combined antiretroviral therapy (cART) who were antiretroviral-naive or had an interruption of cART for at least 12 months, provided that they had at least a baseline cGFR and sAP value as well as two follow-up values, each available for analysis. Similarly, we showed the subpopulation of patients who discontinued TDF. Hepatitis B surface antigen-positive patients were excluded. sAP levels were measured at baseline (range -24–1 weeks) and after 1 (-2–4 weeks), 3 (±6 weeks), 6 (±12 weeks) and 12 (±12 weeks) months of cART and at the same intervals after TDF discontinuation. These intervals corresponded with routine clinical visits. Neither the the differentiation between liver and bone sAP isoenzymes nor γ-glutamyl transferase values were available from the archived data. Serum alanine aminotransferase (sALT) dynamics and the effect of hepatitis C infection on sAP were evaluated in an attempt to detect potential hepatic causes for an increase in sAP. Predefined covariates for linear regression analyses were age, gender, weight, ethnicity, smoking status, diabetes mellitus, systolic blood pressure, baseline cGFR, HIV type-1 (HIV-1) plasma RNA, baseline and nadir $CD4^+$ T-cell count, AIDS status, previous cART, use of TDF, boosted protease inhibitors (PIs) and cotrimoxazole as well as chronically active hepatitis C infection.

All analyses were performed using Stata™ version 9.0. *P*-values were two-sided with statistical significance defined as $P<0.05$.

Results

We included 162 treatment-naive patients starting TDF-containing cART and 495 treatment-naive patients starting TDF-sparing cART, as well as 168 patients reinitiating TDF-containing cART and 193 reinitiating TDF-sparing cART. Baseline characteristics are provided in Table 1. CDC stage III events within 6 months of cART initiation were more frequent in treatment-naive patients than in treatment-experienced patients (17% versus 11%, respectively), but did not differ according to TDF use.

sAP values did not differ between the four groups at baseline ($P=0.9$). TDF use was associated with a significant increase of sAP in both treatment-naive (Figure 1A) and treatment-experienced patients (Figure 1B), but not in patients on TDF-sparing regimes. sALT values remained stable in all subpopulations studied (Figure 1C). ANOVA testing for repeated measurements (with missing values taken forward) confirmed a significant increase in sAP in patients initiating ($P<0.0001$) and reinitiating ($P<0.0001$) TDF-containing cART, but not in patients on TDF-sparing regimes ($P\geq0.1$). Patients with and without chronically active hepatitis C infection showed an identical pattern with a TDF-associated increase in sAP, but not in sALT in contrast to stable sAP values on TDF-sparing regimens (data not shown).

In the 73 individuals discontinuing TDF, the median sAP at the time of TDF discontinuation was similar to the plateau value of the TDF-exposed population (96 U/l) and decreased to baseline values within 1 year (Figure 1D).

In univariable and multivariable linear regression analyses, we found a significant correlation between sAP and TDF use after 6 and 12 months, between sAP, age and HIV-1 RNA after 6 months, as well as between sAP and systolic blood pressure after 12 months (Table 2). There was no correlation between the change of sAP and baseline cGFR (Table 2) or between the change of sAP and the extent of cGFR reduction (>10, >20 or >30 ml/min) associated with TDF medication (data not shown).

PIs-based compared with non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens had lower increases in sAP (data not shown).

Discussion

We document a strong correlation between TDF use and increased sAP in a large observational cohort. This association has already been described in a few small studies [11–13]. The consistency between treatment-naive and treatment-experienced patients and the prompt regression of sAP values to baseline upon TDF discontinuation suggest a causal role of TDF. The increase in sAP is most likely caused by the bone isoenzyme, as cholestatic side effects of TDF have not been described to the best of our knowledge. The lack of changes in sALT in all subpopulations throughout the observation period as well as the consistent increase in sAP in TDF-treated, but not TDF-sparing patients with chronically active hepatitis C infection, provides further evidence against a hepatic origin of the sAP increase. The recent description of lower $CD4^+$ T-cell count increase in TDF-treated patients [14] renders a more pronounced systemic inflammatory response syndrome in TDF-treated patients with a consecutive increase in sAP unlikely. Also, baseline CDC stage and $CD4^+$ T-cell counts as well as the persistent increases of sAP beyond 6 months of therapy argue against a relevant role of opportunistic infections.

Total sAP has been used to monitor bone metabolism and has shown a high correlation with sAP bone isoenzyme levels and a good sensitivity for the detection of

hypophosphataemic osteomalacia [15]. Also, total sAP correlated well with the extent of trauma and healing progression in patients with hip fractures [16]. The sAP bone isoenzyme is a surrogate of osteoblast activity and its increase indicates stimulated bone metabolism [17]. This stimulation might have resulted from TDF causing PRT with renal phosphate and calcium losses. Often, such losses only become apparent when functional tubular parameters, such as the fractional excretion of phosphate are calculated [18]. Renal phosphate and calcium losses can be aggravated by a PRT-related 1′-hydroxylation defect of vitamin D [5], which prevents a compensatory increase in intestinal calcium and phosphate resorption. In this situation, euphosphataemia and eucalcaemia are maintained by compensatory osteolysis, which in turn stimulates regenerative osteoblast activity resulting in the observed increase in sAP. Several studies provide evidence for this pathophysiological concept: high-dose TDF treatment was associated with hypophosphataemia, increased sAP and a rachitic type picture in animal studies with infant rhesus macaques [19]. Also, TDF use was associated with increased calciuria and reduced bone mineral density [10] as well as with phosphaturia and an increase in sAP [12]. Earle *et al.* [11] described a correlation between the increase in sAP and DEXA-confirmed reductions in bone mineral density. Consistently, an association between TDF use and reductions in bone mineral density was documented in a randomized controlled trial including 602 patients [9]. In line with the TDF-related increase in sAP observed in our study, losses in bone mineral density stabilized beyond 6 months of therapy. It is unknown to what extent this stabilization was related to calcium and vitamin D substitution that was introduced at week 144 in this study [20].

TDF-related increases in sAP neither correlated with baseline cGFR nor with TDF-related reductions in cGFR. There is good evidence for the association between TDF-related PRT and bone metabolism; however, no pathophysiological link between PRT and cGFR is known. On the basis of our data, reductions in cGFR seem inadequate to detect TDF-related alterations in bone metabolism.

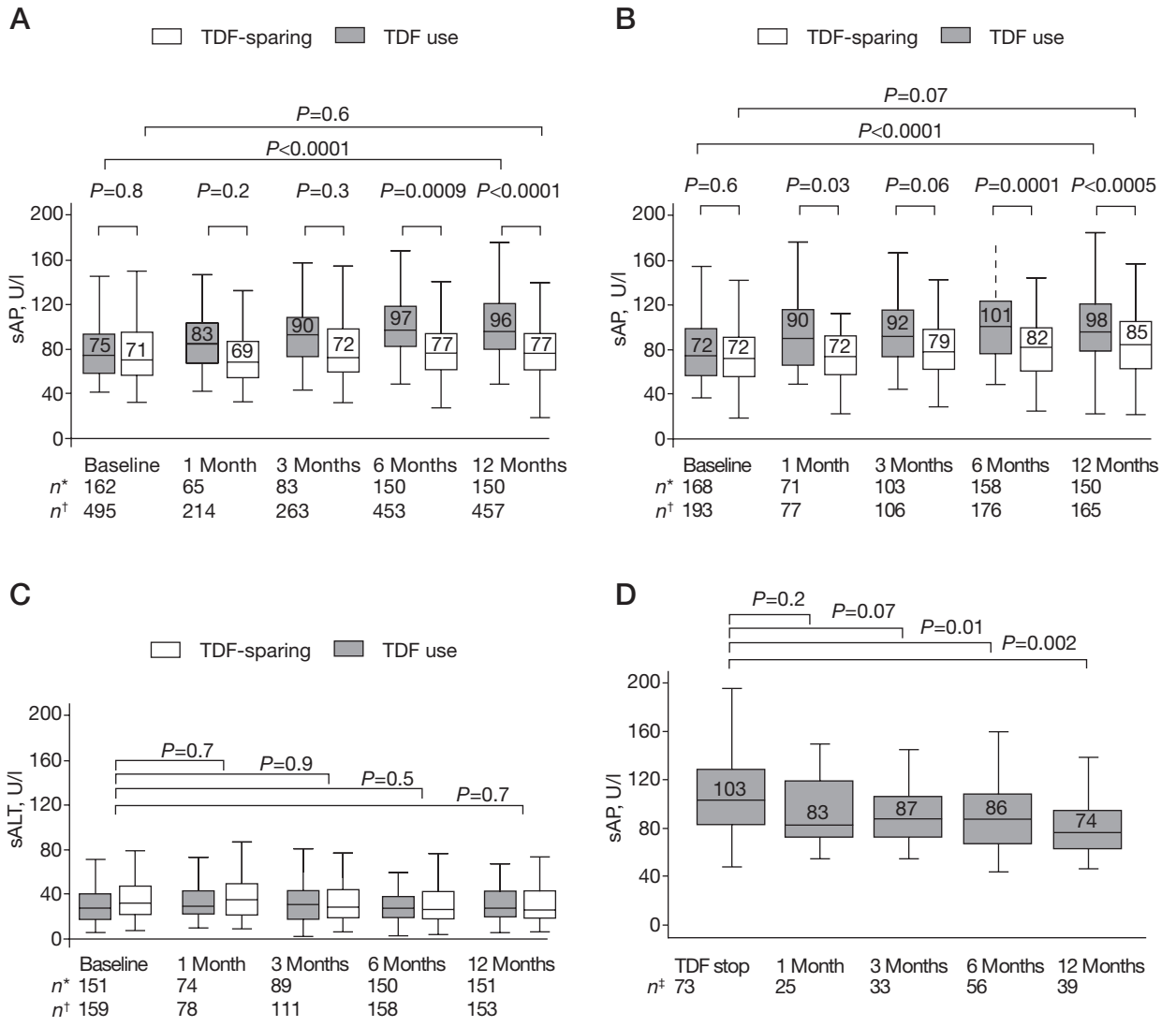
Treatment-experienced patients showed stronger increases in sAP in univariable but not multivariable analyses, which might be because of the higher

Table 1. Baseline characteristics of the four treatment groups at the time of cART (re)initiation

	Treatment group			
Characteristic	Naive to TDF-containing cART	Reinitiating TDF-containing cART	Naive to TDF-sparing cART	Reinitiating TDF-sparing cART
Number of patients, *n*	162	168	495	193
Demographics				
Median age, years (IQR)	39 (34–46)	42 (37–46)	39 (32–45)	41 (36–48)
Female gender, *n* (%)	44 (27)	49 (29)	149 (30)	77 (40)
Median weight, kg (IQR)	69 (61–79)	69 (59–79)	69 (60–78)	68 (58–76)
Caucasian ethnicity, *n* (%)	130 (80)	153 (91)	389 (79)	170 (88)
Vascular risk factors				
Smoking, *n* (%)	78 (48)	80 (48)	211 (43)	118 (61)
Diabetes mellitus, *n* (%)	10 (6)	5 (3)	11 (2)	1 (1)
Arterial hypertension, *n* (%)	35 (22)	40 (24)	118 (24)	57 (30)
HIV status				
Prior AIDS, *n* (%)	33 (20)	31 (18)	77 (16)	37 (19)
Median RNA level, $\log_{10}$ copies/ml (IQR)	4.5 (3.4–5.2)	4.8 (4.0–5.3)	4.9 (4.1–5.4)	4.9 (3.8–5.3)
Median CD4$^+$ T-cell count, cells/μl (IQR)	217 (143–287)	225 (160–277)	207 (126–292)	236 (174–323)
CD4$^+$ T-cell count <200 cells/μl, *n* (%)	70 (43)	65 (39)	236 (48)	70 (36)
Median CD4$^+$ T-cell nadir, cells/μl (IQR)	204 (142–266)	178 (120–231)	190 (121–259)	200 (124–271)
Renal function				
Median cGFR (MDRD), ml/min/1.73 m^2 (IQR)	93 (82–112)	95 (83–109)	96 (85–113)	95 (78–114)
Median cGFR (CG), ml/min (IQR)	101 (84–123)	100 (86–118)	105 (89–123)	104 (83–120)
Comedication				
Cotrimoxazol, *n* (%)	51 (31)	40 (24)	165 (33)	56 (29)
NNRTI, *n* (%)	96 (59)	77 (46)	219 (44)	83 (43)
Nevirapine proportion, *n* (%)	7 (7)	13 (17)	13 (6)	16 (19)
Boosted PI, *n* (%)	63 (39)	99 (60)	208 (47)	85 (53)

cART, combined antiretroviral therapy defined as ≥3 antiretroviral drugs; CG, Cockcroft–Gault formula; cGFR, calculated glomerular filtration rate; IQR, interquartile range; MDRD, modified diet in renal disease formula; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir.

Figure 1. Serum alkaline phosphatase and serum alanine aminotransferase dynamics relative to tenofovir use



Tenofovir (TDF) use, as opposed to TDF-sparing regimens, was associated with a significant increase in serum alkaline phosphatase (sAP) both in **(A)** treatment-naive and **(B)** treatment-experienced patients (re)initiating combined antiretroviral therapy (cART). On the contrary, TDF use did not alter **(C)** serum alanine aminotransferase (sALT) values in treatment-naive or treatment-experienced patients. **(D)** TDF discontinuation was followed by a decrease in sAP reaching baseline values within 1 year. Two-sided *P*-values are provided for comparisons between TDF-containing and TDF-sparing regimens (A & B) and between different time points (A–D), respectively. The boxes indicate median and the 25th and 75th percentile values and the whiskers indicate the upper and lower adjacent values. Values beyond 200 U/l are represented by a dashed whisker. *Number of patients starting TDF-containing cART. †Number of patients starting TDF-sparing cART. ‡Number of patients discontinuing TDF.

proportion of cART-experienced individuals starting TDF compared with the cART-naive individuals (47% versus 25%, respectively). The correlation of sAP with arterial hypertension after 12 months of therapy is unexplained, but might indicate hypertensive vasculopathy leading to bone necrosis.

We have previously shown that TDF and PI use were independently associated with reductions in cGFR [8]. In the current analysis, PI use seemed to counteract TDF-related increases in sAP: PI-based cART as compared with NNRTI-based regimens showed a trend towards lower increases in sAP, with a large heterogeneity between individual PIs. PI-specific effects on osteoclasts and osteoblasts have been described in several *in vitro* studies [21,22]. Their individual effects on TDF-related alterations in bone metabolism should be evaluated in further studies.

We conclude that the TDF-related increase in sAP indicates stimulated bone turnover. Whether this results in actual bone loss in an attempt to compensate for

Table 2. Linear regression analyses for the changes in serum alkaline phosphatase levels after 6 and 12 months of cART

Factor	6 Months of therapy				12 Months of therapy			
	Univariable	*P*-value	Multivariable	*P*-value	Univariable	*P*-value	Multivariable	*P*-value
Age, per 10 years	**5.2 (1.9–8.5)**	**0.002***	**6.3 (2.6–10.1)**	**0.001***	2.4 (-0.7–5.5)	0.1	1.2 (-2.7–5.0)	0.6
Female gender	1.1 (-6.1–8.3)	0.3	1.1 (-7.3–9.4)	0.8	4.7 (-2.1–11.6)	0.2	3.8 (-4.2–11.8)	0.4
Weight, per 10 kg	-0.0 (-2.6–2.5)	1.0	-0.1 (-3.1–3.0)	1.0	-1.0 (-3.4–1.4)	0.4	-1.2 (-4.6–2.1)	0.5
Caucasian ethnicity	-2.7 (-11.7–6.4)	0.6	-6.1 (-16.7–4.4)	0.3	-3.7 (-12.3–4.9)	0.4	-4.5 (-14.6–5.6)	0.4
Smoking	0.9 (-5.9–7.6)	0.3	4.7 (-2.7–12.0)	0.2	-0.7 (-7.1–5.6)	0.8	1.5 (-5.4–8.3)	0.7
Diabetes mellitus	20.0 (0.4–41.6)	0.05	12.6 (-8.4–33.6)	0.2	4.1 (-14.5–22.8)	0.7	-0.3 (-19.0–18.4)	1.0
Systolic BP, per 10 units	-0.4 (-2.4–1.6)	0.7	-1.5 (-3.7–0.7)	0.2	1.5 (-0.4–3.4)	0.1	**2.2 (0.1–4.3)**	**0.04***
Baseline cGFR, per 10 ml/min	-0.2 (-0.7–0.4)	0.5	-0.0 (-0.6–0.6)	0.9	-1.0 (-2.1–0.1)	0.08	-0.8 (-2.3–0.7)	0.3
RNA copies, per $\log_{10}$ copies/ml increase	**-2.7 (-5.3–-0.1)**	**0.04***	-2.3 (-5.2–0.5)	0.1	-2.4 (-4.9–0.0)	0.06	-1.3 (-4.0–1.5)	0.4
$CD4^+$ T-cell count, per 100 cells/μl increase	-0.5 (-2.6–1.7)	0.7	-0.8 (-5.9–4.3)	0.8	1.2 (-0.9–3.2)	0.3	-2.5 (-7.2–2.3)	0.3
$CD4^+$ T-cell nadir, per 100 cells/μl increase	-0.7 (-3.3–1.9)	0.6	-0.2 (-6.2–5.7)	0.9	1.9 (-0.6–4.4)	0.1	4.5 (-1.1–10.1)	0.1
Prior AIDS status	-5.0 (-14.2–4.1)	0.3	-6.7 (-16.3–2.9)	0.2	-7.3 (-15.8–1.4)	0.1	-4.4 (-13.4–4.5)	0.3
cART experience	2.6 (-4.3–9.5)	0.5	-0.2 (-7.7–7.4)	1.0	**7.2 (0.6–13.7)**	**0.03***	4.3 (-2.7–11.4)	0.2
Tenofovir	**13.0 (6.0–19.9)**	**<0.001***	**11.1 (3.8–18.4)**	**0.003***	**20.1 (13.5–26.6)**	**<0.001***	**18.7 (11.8, 25.6)**	**<0.001***
Boosted protease inhibitor	-5.1 (-11.8–1.6)	0.1	-3.9 (-10.8–3.0)	0.3	-5.3 (-11.7–1.1)	0.1	-4.8 (-11.3–1.6)	0.1
Cotrimoxazole	-0.6 (-8.0–6.7)	0.9	1.3 (-7.3–10.0)	0.8	-5.7 (-12.6–1.2)	0.1	-0.7 (-8.7–7.3)	0.9
Hepatitis C virus infection	-6.4 (-14.3–1.6)	0.1	-6.9 (-15.6–1.8)	0.1	-4.7 (-12.4–2.9)	0.2	-3.0 (-11.2–5.3)	0.5

Coefficients with 95% confidence intervals are shown. The multivariable analysis includes all parameters shown in the table. *Statistically significant ($P<0.05$) variables are in bold. BP, blood pressure; cART, combined antiretroviral therapy defined as ≥3 antiretroviral drugs; cGFR, calculated glomerular filtration rate.

TDF-related excessive renal phosphate losses, remains to be proven. We believe, however, that the available data together with the plausible pathophysiology raise concerns about the long-term safety of TDF, which should be addressed in further studies.

For clinical practice, we suggest screening TDF-treated patients for PRT by measuring phosphataemia, the fractional excretion of phosphate, proteinuria and glucosuria [18]. In patients with drug-related PRT, the quantification of bone metabolism and bone mineral density should be considered, particularly in the presence of risk factors for osteoporosis. On the basis of current data, no recommendations can be given about when to discontinue TDF or to supplement calcium and vitamin D in order to prevent bone disorders.

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Disclosure statement

The authors declare no competing interests.

Additional file

An additional file listing the members of the SHCS can be accessed via the Volume 13 Issue 8 contents page for *Antiviral Therapy*, which can be found at www.intmedpress.com (by clicking on 'Antiviral Therapy' then 'Journal PDFs').

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